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Transducer protein HtrI controls proton movements in sensory rhodopsin I

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Abstract

Sensory rhodopsin I (SR-I λ_{max} 587 nm) is a phototaxis receptor in the archaeon *Halobacterium salinarium*. Photoisomerization of retinal in SR-I generates a long-lived intermediate with λ_{max} 373 nm which transmits a signal to the membrane-bound transducer protein HtrI. Although SR-I is structurally similar to the electrogenic proton pump bacteriorhodopsin (BR), early studies showed its photoreactions do not pump protons, nor result in membrane hyperpolarization. These studies used functionally active SR-I, that is, SR-I complexed with its transducer HtrI. Using recombinant DNA methods we have expressed SR-I protein containing mutations in ionizable residues near the protonated Schiff base, and studied wild-type and site-specifically mutated SR-I in the presence and absence of the transducer protein. UV-Vis kinetic absorption spectroscopy, FT-IR, and pH and membrane potential probes reveal *transducer-free* SR-I photoreactions result in vectorial proton translocation across the membrane in the same direction as that of BR. This proton pumping is suppressed by interaction with transducer which diverts the proton movements into an electroneutral path. A key step in this diversion is that transducer interaction raises the pK_a of the aspartyl residue in SR-I (Asp76) which corresponds to the primary proton-accepting residue in the BR pump (Asp85). In transducer-free SR-I, our evidence indicates the pK_a of Asp76 is 7.2, and ionized Asp76 functions as the Schiff base proton acceptor in the SR-I pump. In the SR-I/HtrI complex, the pK_a of Asp76 is 8.5, and therefore at physiological pH (7.4) Asp76 is neutral. Protonation changes on Asp76 are clearly not required for signaling since the SR-I mutants D76N and D76A are active in phototaxis. The latent proton-translocation potential of SR-I may reflect the evolution of the SR-I sensory signaling mechanism from the proton pumping mechanism of BR.

Keywords: Sensory rhodopsin I; Proton pump; Transducer; HtrI; Bacteriorhodopsin

1. Introduction

Halobacterium salinarium (formerly known as *H. halobium*) is a motile archaeon capable of photosensory transduction and photoenergy conversion by a family of retinylidene proteins. Two, sensory rhodopsin I (SR-I, [1,2]) and sensory rhodopsin II (SR-II, [3]) are phototaxis receptors which modulate cell swimming behavior. The other two are light-driven electrogenic pumps: bacteriorhodopsin (BR, a proton pump, [4]) and halorhodopsin (HR, a chloride pump, [5]). The archaeal rhodopsins resemble structurally the visual pigments of higher organisms each consisting of a single polypeptide which folds into

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seven membrane-spanning α-helical segments forming an internal pocket where the chromophore retinal is bound [6,7].

2. SR-I photoreceptor

A structural model of the packing and orientation of SR-I helices is suggested from comparison of its sequence [8] with that of BR, for which atomic to near-atomic resolution structural information has been obtained from cryoelectron diffraction [9]. In the model, the seven α-helical columns form an annular structure in the membrane, creating an interior cavity in which the retinal is bound via a protonated Schiff base linkage [10] to the ε-amino group of Lys205. Several lines of evidence support the use of the BR structure as a first approximation to that of

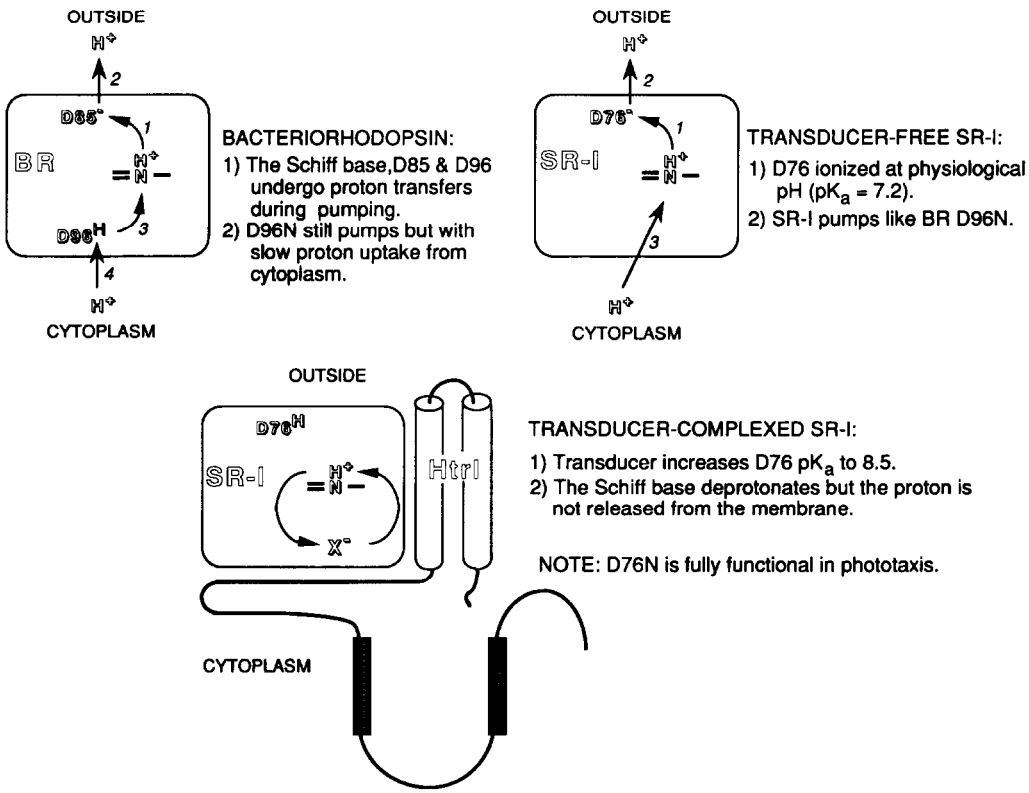

Fig. 1. Model for proton pumping by SR-I and its inhibition by HtrI. BR (upper left): The proton path is based on work reviewed in Ref. [35–37]. Photoisomerization of retinal causes transfer of the Schiff base proton to Asp85 which is ionized at neutral pH. Asp85 is part of a proton release path to the extracellular medium. Reprotonation of the Schiff base nitrogen occurs from Asp96 which is protonated at the beginning of the pumping cycle and reprotonates from the cytoplasm in the latter half of the cycle. BR still pumps but at a reduced rate if Asp96 is replaced by asparagine. Neutralization of Asp85 by mutation to asparagine blocks the pump and results in proton release to the cytoplasm [38]. HtrI-free SR-I (upper right): Asp76 is in the corresponding position as Asp85 in BR, and is ionized in mildly alkaline conditions. The alkaline form of SR-I (SR_{550}) exists in a proton-dependent equilibrium with a form in which Asp76 is neutral (SR_{590}). SR_{550} is a light-driven electrogenic pump [30] which ejects protons from the cell via Asp76 and a slow cytoplasmic return path similar to that of BR D96N. HtrI-complexed SR-I (bottom): The pK_a of Asp76 is increased to 8.5 by interaction with transducer and the 590 nm form is therefore stabilized. In addition to raising the pK_a of Asp76, HtrI prevents the equilibration of released protons with the bulk aqueous phase [25]. The proton is depicted as released toward the cytoplasmic side of SR-I because SR_{590} (transducer-free) apparently releases protons to the cytoplasmic side, since release to the medium after a light flash is detected only if right-side-out vesicles are disrupted by low salt or detergent (K.D. Olson and J.L. Spudich, unpublished results).

SR-I, especially in the retinal-binding cavity, which exhibits 81% conservation of BR residues [8] and similar electrostatic and hydrophobic interactions with the chromophore [11].

3. SR-I signaling and the photocycle intermediate S_{373}

SR-I controls swimming behavior of the cells by modulating the frequency of reorientation ('reversals') of their swimming direction. Orange light (590 nm) generates attractant signals that suppress reversals, whereas near-UV light (370 nm) generates repellent signals that induce reversals [2]. Several events in the receptor signaling process have been elucidated. Photon absorption by SR-I (λ_{max}, 587 nm) causes isomerization around the C13=C14 double bond of the retinal chromophore [12] which is essential for receptor activation [13]. The photoisomerization energy is transferred to the protein in a process requiring steric interaction between the retinal C13 methyl group and protein [11], and triggers a transition within 1 ms to an activated conformation of the molecule, which via a signal transduction pathway suppresses flagellar reversals. The activation process is accompanied by a shift in the absorption spectrum to the near UV ($\lambda_{max} = 373$ nm), indicative of deprotonation of the Schiff base [14,15]. In the dark the activated species, S_{373}, decays thermally to the prestimulus state with a $t_{1/2} \simeq 750$ ms at room temperature. Photoexcitation of S_{373} more rapidly converts it back to SR_{587} and this process causes repellent (reversal) responses [2]. Hence, deprotonation accompanies attractant signaling and light-induced reprotonation of the Schiff base, repellent signaling.

4. Sensory rhodopsin I transducer HtrI

Biochemical studies of mutants defective in signaling identified another intrinsic membrane protein involved in the transduction of SR-I signals [16,17]. Partial sequence from the proposed transducer protein now designated HtrI (*H*alobacterial *t*ransducer for sensory *r*hodopsin *I*), allowed the identification and cloning of its gene, *htrI* [18].

The deduced sequence of HtrI predicts two transmembrane helices near the N terminal that would anchor the protein to the membrane (Fig. 1). Beyond this hydrophobic region of 46 residues, the remainder of the protein (536 amino acid residues total) is hydrophilic. The C-terminal 270 residues contain a region homologous to the signaling domains of eubacterial chemotaxis transducers (e.g., *E. coli* Tsr protein [19]) flanked by two regions homologous to the methylation domains of this transducer family. The protein differs from *E. coli* Tsr in that it does not have an extramembranous ligand-binding domain and that it has a more extended cytoplasmic region. The 220 residues linking the putative transmembrane domain and the signaling and methylation domain are not homologous to any protein sequence available.

When we cloned and sequenced the *htrI* gene, it was found to lie immediately upstream of the gene encoding the SR-I apoprotein or opsin [*sopI* (sensory opsin I; [8])]. The *sopI* gene initiator codon overlaps the termination codon of *htrI*, and the *htrI–sopI* pair is preceded by a putative promoter region [18] and the two genes are co-transcribed [20]. The two proteins are expressed in *H. salinarium* following transformation with a plasmid carrying the putative promoter region and the gene pair, and they restore phototaxis in a mutant containing a deletion in the *htrI–sopI* region [18,20]. Furthermore, an *htrI* deletion shows that the region encoding the methylation and signaling domain of HtrI is required for the restoration of SR-I phototaxis [21].

5. Evidence for an SR-I / HtrI molecular complex

Physical proximity of HtrI to SR-I was first indicated by transfer of radioactive retinal from SR-I specifically to HtrI during treatment of membranes with a reducing agent [16]. Recently, expression studies have demonstrated that in native membranes HtrI alters SR-I photochemical reactions [22,23,25] and their pH sensitivity [22,25]. HtrI also limits the accessibility of the retinylidene Schiff base to aqueous reagents (hydroxylamine, sodium hydroxide) (summarized in [24]) and prevents the release of protons into the medium upon deprotonation of the Schiff base [25]. It is not known whether the HtrI transmembrane or cytoplasmic regions or both con-

tain the sites of interaction with the receptor protein. The interactions responsible for HtrI modulation of SR-I photoreactions discussed in the following section, do not require the signaling and methylation domains as shown by deletion of the C-terminal half of HtrI [21].

6. Transducer-control of proton transfers in SR-I

SR-I is structurally similar to the electrogenic proton pump BR, and like BR, SR-I appears to deprotonate and later reprotonate the Schiff base nitrogen during its photocycle. Nevertheless, early studies showed its photoreactions do not translocate protons nor result in membrane hyperpolarization [1,26,27]. These studies used functionally active SR-I, that is, SR-I complexed with its transducer HtrI. Using plasmid-directed expression of an SR-I apoprotein synthetic gene [28], we have produced transducer-free SR-I in *H. salinarium* membranes and SR-I containing mutations in ionizable residues near the protonated Schiff base. Studies of mutant membranes have led to a series of findings which reveal a fundamental similarity between SR-I and BR despite their different functions [22,25,29,30]. The picture emerging from UV-Vis kinetic absorption spectroscopy, FT-IR and pH and membrane potential probes is as follows (Fig. 1): Photoreactions of transducer-free SR-I do vectorially translocate protons across the membrane in the same direction as BR. In SR-I this pumping is suppressed by interaction with transducer which diverts the proton movements into an electroneutral path. A key step in this diversion is that transducer interaction raises the pK_a of the aspartyl residue in SR-I (Asp76) which corresponds to the primary proton-accepting residue in the BR pump (Asp85). The main observations supporting this interpretation are:

(1) Removal of transducer alters the photochemical reactions of the receptor so that the thermal decay of S_{373}, which requires reprotonation of the retinal attachment site in the photoactive center of the protein, becomes highly pH-sensitive (reprotonation proceeds more rapidly in proportion to the proton concentration; [22]). This observation was the first indication of a proton path from the medium to the SR-I photoactive site.

(2) In transducer-free SR-I, formation and decay of S_{373} are accompanied by release and uptake, respectively, of protons in the medium [25]. Transducer binding blocks this proton exchange [25,31]. Our proton release measurements were greatly facilitated by the finding that SR-I is overproduced when its C-terminal region is truncated [32].

(3) The proton release and uptake is vectorial (from the cytoplasmic to the extracellular side of the membrane) if a residue of $pK_a \approx 7.2$ is deprotonated, which shifts the SR-I absorption from 590 nm to 550 nm [30]. Transducer interaction raises the pK_a of this residue to ≈ 8.5 [31].

(4) Fourier transform infrared spectroscopy shows D76, the aspartyl residue in SR-I in a corresponding position as the aspartyl proton-acceptor near the BR chromophore is neutral in transducer-complexed SR-I [29]. Furthermore, the properties of SR-I and D76N indicate Asp76 is the residue with $pK_a \approx 7.2$ in (3). In particular D76N does not exhibit the transition to a 550 nm form in the presence or absence of HtrI [tested up to pH 8.7 [33]] nor does it pump protons at pH 7.0. One-photon pumping (Fig. 1) is referred to here. D76N might be expected to exhibit two-photon inverted pumping as shown for transducer-free SR-I (R.A. Bogomolni, personal communication).

The latent proton-translocation capacity of SR-I may reflect the evolution of the SR-I sensory signaling mechanism from the proton pumping mechanism of BR. An important point to emphasize is that pumping does not occur in the presence of HtrI and Asp76 is not necessary for phototaxis [29]. Thus, the proton pumping appears to be an evolutionary vestige in SR-I.

An attractive hypothesis is that the electroneutral proton path in transducer-complexed SR-I is important to activation of the transducer. Two alternative models for tight coupling of HtrI to proton transfer sites in SR-I are: (1) Direct coupling, i.e., an HtrI residue(s) participates in the proton circulation during the receptor photocycle. (2) Indirect coupling, i.e. receptor proton transfer reactions couple via an allosteric mechanism to a receptor/transducer interaction site. In both of these cases, discussed in more detail in Ref. [34], critical proton transfers are predicted. The alternative is that the nature of the particular proton acceptor from the Schiff base is not critical, and that the proton transfer will be able to be

diverted to another acceptor without loss of signaling function.

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